Dopaminergic abnormalities following traumatic brain injury

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Abstract

Traumatic brain injury can reduce striatal dopamine levels. The cause of this is uncertain, but is likely to be related to damage to the nigrostriatal system. We investigated the pattern of striatal dopamine abnormalities using ^{123}I-Ioflupane SPECT scans and their relationship to nigrostriatal damage and clinical features. We studied 42 moderate-severe traumatic brain injury patients with cognitive impairments but no motor parkinsonism signs and 20 healthy controls. ^{123}I-Ioflupane scanning was used to assess dopamine transporter levels. Clinical scan reports were compared to quantitative dopamine transporter results. Advanced MRI methods were used to assess the nigrostriatal system, including the area through which the nigrostriatal projections pass as defined from high-resolution Human Connectome Data. Detailed clinical and neuropsychological assessments were performed. Around 20% of our moderate-severe patients had clear evidence of reduced specific binding ratios for the dopamine transporter in the striatum measured using ^{123}I-Ioflupane SPECT. The caudate was affected more consistently than other striatal regions. Dopamine transporter abnormalities were associated with reduced substantia nigra volume. In addition, diffusion MRI provided evidence of damage to the regions through which the nigrostriatal tract passes, particularly the area traversed by dopaminergic projections to the caudate. Only a small percentage of patients had evidence of macroscopic lesions in the striatum and there was no relationship between presence of lesions and dopamine transporter specific binding ratio abnormalities. There was also no relationship between reduced volume in the striatal sub-regions and reduced dopamine transporter specific binding ratios. Patients with low caudate dopamine transporter specific binding ratios show impaired processing speed and executive dysfunction compared to patients with normal levels. Taken together, our results suggest that the dopaminergic system is affected by a moderate-severe traumatic brain injury in a significant proportion of patients, even in the absence of clinical motor parkinsonism. Reduced

dopamine transporter levels are most commonly seen in the caudate and this is likely to reflect the pattern of nigrostriatal tract damage produced by axonal injury and associated midbrain damage.

Introduction

Traumatic brain injury (TBI) can damage the dopaminergic system. Animal models of TBI show both dopaminergic cell loss (van Bregt *et al.*, 2012) and biochemical disturbance of the dopaminergic system. Biochemically, a brief transitory rise in dopamine levels throughout the brain (Huger and Patrick, 1979; McIntosh *et al.*, 1994; Massucci *et al.*, 2004; Kobori *et al.*, 2006) is followed by a functional hypodopaminergic state (Wagner *et al.*, 2005b). Human neuropathology studies also show head injuries cause gross and microscopic changes to the substantia nigra (Smith *et al.*, 2013).

The dopaminergic cell bodies reside in two main nuclei in the midbrain, the substantia nigra and the ventral tegmental area (Bjorklund and Dunnett, 2007). These nuclei are therefore susceptible to midbrain and brainstem injuries that are common after TBI, particularly in those patients with poor outcome (Adams *et al.*, 1989). The ascending dopaminergic neurons project to subcortical and cortical areas, with striatal projections travelling through the nigrostriatal tract. These ascending fibres are likely to be susceptible to damage from shearing forces generated by trauma, but this has not previously been investigated. Computational models show high strain across the midbrain when subjected to external forces as the brain pivots in this region, thereby providing a biomechanical explanation for susceptibility to injury in this area (Zhang *et al.*, 2001).

Striatal dopamine abnormalities can be assessed *in vivo* using single-photon emission computed tomography (SPECT) imaging to measure dopamine transporter (DaT) expression (Yan *et al.*, 2002; Wagner *et al.*, 2005a; Wilson *et al.*, 2005; Wagner *et al.*, 2009). This imaging technique is widely used in the diagnosis of Parkinson's disease (Marek *et al.*, 1996; Wenning *et al.*, 1998; Benamer *et al.*, 2003). DaT is located on presynaptic dopaminergic neurons (see Fig. 1.A.) with extracellular dopamine levels and neural activity regulating its

expression (Gulley and Zahniser, 2003). DaT levels are reduced secondary to dopaminergic cell loss or to a compensatory down-regulation because of reduced dopamine levels. In humans, two small imaging studies have shown reduced overall striatal DaT binding using SPECT and PET after TBI (Donnemiller *et al.*, 2000; Wagner *et al.*, 2014).

The striatum is sub-divided into the caudate, putamen and nucleus accumbens, which have distinct functions. In Parkinson's disease, motor abnormalities are associated with early DaT reductions in the posterior putamen (Guttman *et al.*, 1997; Ma *et al.*, 2002). As abnormalities progress anteriorly to involve the caudate, patients show more cognitive impairment (Ekman *et al.*, 2012). Following a single TBI patients rarely show motor parkinsonism features (Krauss and Jankovic, 2002), but commonly show cognitive impairments that overlap with those seen in Parkinson's disease (Kehagia *et al.*, 2010). This could be explained by a non-uniform pattern of DaT abnormalities within the striatum.

Here we extend this previous work in a large study using a multi-modal imaging approach to explore the location of dopaminergic abnormalities within the striatum and its relation to structural damage within the nigrostriatal system. ^{123}I-Ioflupane SPECT was used to calculate DaT specific binding ratios (SBRs) within the striatum. Advanced diffusion and volumetric MRI methods were used to investigate post-traumatic damage to the substantia nigra and ascending nigrostriatal fibres defined using high-resolution Human Connectome Project (HCP) data (Van Essen *et al.*, 2013). The following hypotheses were tested: (1) DaT levels are reduced following moderate-to-severe TBI; (2) DaT levels in areas of the striatum related to cognitive functioning (caudate) are affected more than areas associated with motor functioning (putamen); (3) evidence of structural damage to the substantia nigra and/or the areas through which the ascending nigrostriatal fibres pass relate to reduced striatal DaT levels; (4) alterations in striatal DaT levels relate to cognitive impairments; in particular, processing speed, attention and executive functions.

Materials and methods

The study was approved by the West London and GTAC NRES Committee (14/LO/0067). All participants provided informed consent.

Eligibility, Groups and Screening

Patients were recruited from specialist TBI clinics in London, UK. Inclusion criteria were: (1) aged 20-65; (2) history of single moderate-severe TBI (Mayo classification (Malec *et al.*, 2007)) at least 3 months prior; (3) subjective complaint of cognitive difficulties by participant, treating clinician, or caregiver. Exclusion criteria included: (1) neurological or psychiatric illness diagnosed prior to TBI; (2) drug or alcohol addiction; (3) positive urine drug screen (4) contraindication to MRI or SPECT; (5) contraindication to methylphenidate use (as this study formed part of a clinical trial, which will be reported separately); (6) clinical evidence of motor signs of parkinsonism as assessed by a Neurologist. Following baseline neuropsychological assessment, SPECT and MRI scans; the TBI patients were randomized into a double-blind placebo-controlled cross-over study of methylphenidate, which is the subject of a separate publication (in preparation).

A group of 20 age-matched, healthy control participants were used for the SPECT and MRI analyses. They were in good general health with no history of significant illness, and ineligible if they met any exclusion criteria above.

A screening visit involved medical history and physical examination including the Unified Parkinson's disease rating scale (UPDRS) motor subscale (Goetz *et al.*, 2007). None of the patients screened had evidence of motor parkinsonism.

Study Design

A cross-sectional study of 42 TBI patients compared to 20 controls was performed. All participants had ^{123}I-Ioflupane SPECT, MRI including volumetric T1 and diffusion MRI, and detailed neuropsychological testing.

Study Procedures

Study procedures were carried out at the Hammersmith Hospital, Imperial College London (London, UK) and Charing Cross Hospital (London, UK).

^{123}I-Ioflupane Single Photon Emission Computed Tomography

Before administration of ^{123}I-Ioflupane, patients received potassium iodide tablets (2x60mg) to minimize radiation exposure to the thyroid gland. One hour later, a bolus intravenous injection of ^{123}I-Ioflupane (GE Healthcare Ltd) was administered to each individual (mean activity 185MBq). SPECT images for all subjects were acquired using the same dual-headed gamma camera (Symbia T16, Siemens Healthcare) at 180 minutes post-injection with LEHR collimators, 128x128 matrix, 1.45 zoom, 128 projections, and 30 seconds per projection.

Magnetic resonance imaging

All participants had a T1-weighted high-resolution MPRAGE scan (160 1-mm-thick transverse slices, TR = 2300 ms, TE = 2.98 ms, FA = 9°, in-plane resolution = 1 x 1mm, matrix size = 256 x 256, field of view = 25.6 x 25.6 cm). Diffusion-weighted images were acquired along 64 non-colinear directions with b=1000s/mm^2 and four averages with b=0s/mm^2, with TE/TR 103/9,500ms, 64 contiguous slices, FoV 256 mm, and voxel size 2mm^3.

Neuropsychological assessment

A previously described neuropsychological battery assessed cognitive domains often observed to be impaired after TBI (Kinnunen *et al.*, 2011) (Table 1). In addition, questionnaires were used to assess apathy (the Lille Apathy Rating Scale (Sockeel *et al.*, 2006)), fatigue (the Visual Analogue Scale of Fatigue (Lee *et al.*, 1991)), and changes in behaviour post-injury (both the 'self' and 'family' versions of the Frontal Systems Behaviour Scale (Grace and Malloy, 2001)). Functional outcome was assessed in patients using the Glasgow Outcome Scale-Extended (GOS-E) (Wilson *et al.*, 1998) (Supplementary Table 2).

Image Analysis (Fig. 1)

^{123}I-Ioflupane Single Photon Emission Computed Tomography

Acquired data were reconstructed with an OSEM based iterative algorithm (HybridRecon, HERMES Medical Solutions; Stockholm, Sweden) including corrections for attenuation, scatter, and resolution (Fig. 1.A.i.). The reconstructed SPECT images were co-registered with their corresponding native T1 MRI image. T1 images were segmented into grey and white matter using SPM12, and warped to an average group template using a diffeomorphic

nonlinear registration (DARTEL) (Ashburner, 2007). Templates were registered to Montreal Neurological Institute 152 (MNI) space. Then the individual flow-fields and template transformation from DARTEL were applied to the SPECT images to produce MNI space images, without modulation.

The specific binding ratio (SBR) was calculated for the whole striatum and the caudate, putamen and nucleus accumbens separately. The SBR was calculated using the formula ([region of interest counts − background counts]/background counts) where the background counts were calculated from the occipital cortex, an area of low DaT expression. The SBR is a semi-quantitative technique that takes into account variations in non-specific uptake of ^{123}I-Ioflupane between individuals. It makes the assumption that the non-specific uptake is equivalent throughout the brain.

All SPECT images were also independently reported by two nuclear medicine Consultants following a predefined ranking scale of normal, abnormal or indeterminate. Any discrepancies between reports were resolved by consensus opinion to give a single result per scan.

Lesion Segmentation

In patients, lesions apparent on T1 MRI were manually segmented and excluded from all imaging analyses (Fig. 2). In addition, lesion volumes within the striatum and frontal lobe were measured to test for a relationship between the presence and size of lesions and striatal SBR.

Voxel-Based Morphometry (VBM)

For cross-sectional VBM, T1 images were segmented into grey and white matter, and warped to MNI space (as above) but including modulation by the Jacobian determinants (JDs)

derived from DARTEL (Fig. 1B). The normalized segmentations were then smoothed (4mm FWHM) and masked (group mean p>0.2). Total grey and white matter tissue volumes, and intracranial volume (ICV), were calculated using SPM12. A mask of the substantia nigra(Keuken *et al.*, 2014) and striatum (derived from the Harvard-Oxford probabilistic anatomical atlas within FSL) in MNI space were thresholded at >50% probability and used to calculate volumes for these structures in each individual by overlaying on the individual normalised volumetric images.

Diffusion Tensor Imaging (DTI)

Diffusion-weighted images were preprocessed using standard methods (Kinnunen *et al.*, 2011) and tensor-based registration performed using DTI-TK (Zhang *et al.*, 2007) (Fig. 1C). Maps of fractional anisotropy (FA) and mean diffusivity (MD) were generated from MNI space tensor images. A map of the area through which the nigrostriatal tract passes, as well as nigro-caudate, nigro-putamen and nigro-nucleus accumbens components of this tract, was created using 100 subjects from the HCP (Van Essen *et al.*, 2013). Fibre-tracking was performed in both directions between the left and right substantia nigra and the corresponding nucleus accumbens, putamen and caudate using the MRtrix package (Tournier *et al.*, 2012). 3000 tracks were performed with maximum angle between successive steps limited to 45 degrees. A probability mask based on the percentage of tracks passing through each voxel was created for each individual and thresholded at 5%. All masks were binarised and masks from the two directions of tracking were combined. A group probabilistic mask that was then thresholded at 25% to create a group mask.

Statistical Analyses

Group characteristics were compared using independent sample t-tests (for age) and Fisher's exact test (gender). Voxelwise cross-sectional comparison of ^{123}I-Ioflupane SBR was performed using nonparametric permutation tests (Nichols and Holmes, 2002) in FSL (5000 permutations). Age was added as a nuisance covariate. All results were cluster corrected using threshold-free cluster enhancement (Smith and Nichols, 2009) with family-wise error rate of $p<0.05$. For the ^{123}I-Ioflupane SBR region of interest (ROI) analysis, a linear mixed-effects model was used to assess group differences within the sub-divisions of the striatum (caudate, putamen and nucleus accumbens). Group and ROI were defined as fixed effects, whereas subject was defined as a random effect to model variability in subject intercepts. Age was included as a co-variate. Post-hoc unpaired sample t-tests were used to investigate any significant main effects or interactions. To remove the variance in SBR that relates to age from these post hoc tests, residuals from a linear regression of age on region SBR were used, then differences between patients and controls were assessed per region: caudate, putamen and nucleus accumbens. A similar linear mixed-effects model was used for analysis of diffusion metrics using multiple ROIs (nigro-caudate, nigro-putamen and nigro-nucleus accumbens tracts). Age was included as a co-variate. For volumetric analysis, volumes were corrected for total intracranial volume to remove differences caused by head size, and age was added as a nuisance co-variate. Substantia nigra volumes were compared using an unpaired sample t-test. To assess the sub-divisions of the striatum a mixed-effects model was used followed by post-hoc unpaired sample t-tests. To explore the relationship between the structural measures (substantia nigra volume and tract diffusivity measures) and ^{123}I-Ioflupane SBR we used linear regression to predict striatal sub-region SBR with the structural measure and group (i.e. patient or control) as explanatory variables and age (and intracranial volume for volumetric measures) as nuisance variables.

Differences in performance on neuropsychological tests and questionnaires for patients and controls were investigated with the use of Wilcoxon Rank Sum Tests for independent samples and corrected for multiple comparisons using the false discovery rate method. Subjects greater than 3 standard deviations from the mean on tests were considered outliers and removed from further analysis. This applied to four subjects in the Choice Reaction Task (one control removed for prolonged reaction time, two patients for excessive misses and one patient for excessive errors), one control in the People's Test and two patients in the Trail Making task. One control and one patient were also removed from the Choice Reaction Task analysis due to faulty equipment on the day of testing.

To explore the relationship between neuropsychological performance and ^{123}I-Ioflupane SBR, patients were split based on their clinical ^{123}I-Ioflupane scan report and also based on the quantitative analysis using both one standard deviation and two standard deviations below the control mean for caudate, putamen, nucleus accumbens and whole striatum.

Statistical analysis and graph illustration were performed using R version 3.3.2 (http://www.R-project.org/).

Results

Baseline characteristics

Forty-two TBI patients and 20 healthy controls were enrolled. Patients comprised 36 males (86%) with mean age 39.8 years (standard deviation 11.7, range 20-65). Median time since injury was 34 months (range 6-366 months) (Supplementary Table 1). There was no significant age difference with the healthy controls (16 males (80%), mean age±s.d. 40.2±12.9, range 21-61). Thirty patients had one or more focal lesions (Fig. 2). The majority

of lesions appeared in frontal and/or temporal lobes. No patients had focal lesions in the substantia nigra. Six patients had lesions involving the striatum. These lesions were all very small with the largest lesion load for a single patient comprising just 5% of the total striatal volume (range 0.8-5.0%, mean 3.1%). Lesioned areas were masked out of further analyses.

Clinical reports of ^{123}I-Ioflupane scans

A third of patients (14/42) had abnormal ^{123}I-Ioflupane scans on clinical reporting (Fig. 3 and Fig. 4). No controls had scans reported as abnormal on clinical classification. Three controls and three patients were clinically classified as indeterminate. The pattern of loss of uptake was most often described as patchy. At the individual level, abnormalities were sometimes apparent throughout the striatum including the putamen and caudate, although these were often inconsistent across individuals.

Patients show reduced ^{123}I-Ioflupane specific binding ratios in the caudate

Across the whole group, whole-brain voxelwise analysis showed patients had significantly lower SBR in the anterior striatum, with significant differences found exclusively in the caudate (Fig. 5A and 5B). SBR was not reduced in the putamen across the group. We then used a region of interest approach to explore the effect of TBI on striatal sub-regions (Fig. 5C). A mixed-effects model including the caudate, putamen and nucleus accumbens showed a region of interest by group interaction ($F(2,120)=3.08$, $p=0.0495$). This was driven by a significantly lower SBR in the caudate for patients compared to controls ($t(40.5)=-3.48$, $p=0.001$), with a difference that approached significance in the putamen ($p=0.052$) and no difference in the nucleus accumbens ($p=0.236$). The effect size for differences in caudate

SBR was large (Cohen's d=0.92, 95% CI [0.35, 1.49]), but small in the putamen (Cohen's d=0.50, 95% CI [-0.05, 1.05]) and nucleus accumbens (Cohen's d=0.32, 95% CI [-0.23, 0.86]).

Classifying individual patients ^{123}I-Ioflupane scans

Clinical classification was compared with quantitative results (Fig. 4). To allow comparison between the two sets of results scans clinically classified as 'indeterminate' were grouped with 'normal' scans. In addition, we categorised patients on quantitative results into normal and abnormal using two criteria; (i) greater than 1 standard deviation (s.d.) below the mean SBR in the control group; and (ii) greater than 2 s.d. below the mean SBR. The more stringent criteria (2 s.d.) gave consistent results to the clinical classification with no false-positives in the control population. Using this approach, 8 patients were classified as abnormal based on striatal SBR and 7 based on caudate or putamen SBR. All these patients were also classified as abnormal on clinical assessment. The less stringent criterion (1 s.d.) led to three false positives in the controls and as expected more patients classified as abnormal (16 in the striatum, 18 in the caudate and 15 in the putamen). Classification based on the whole striatum (2 s.d.) showed the greatest concordance with the clinical classification (Cohen's Kappa=0.67). Whole striatum (1 s.d.) also showed reasonable agreement with the clinical classification (Cohen's Kappa=0.55), albeit with more 'false positives' classified in the quantitative group.

Processing speed and functional outcome relate to ^{123}I-Ioflupane scan abnormalities

Over the whole group, TBI patients showed impaired performance in a range of cognitive domains, including tests of processing speed, memory and executive function when compared with the control group (Table 1). Patients also reported significantly increased apathy, fatigue and executive dysfunction compared to controls, a finding that was corroborated on caregiver questionnaires (Supplementary Table 2).

Patients with significantly reduced caudate DaT levels as defined using the 2 s.d. criteria showed impairments in measures of processing speed of varying complexity compared to patients with normal caudate DaT levels. Specifically, abnormal patients were significantly slower on simple measures of processing speed including the Trail Making Test A (W=177, p=0.027) and Stroop Colour Naming & Word Reading Composite Score (W=181.5, p=0.045) as well as on the more complex Stroop Inhibition-Switching Task (W=189.5, p=0.022) (Supplementary Fig. 3). Functional outcomes as defined by the GOS-E were also worse in these patients (W=66, p=0.033). There were no differences in neuropsychological measures using the less stringent classification criterion (1 s.d.). Patients defined as abnormal on the clinical report did not show any difference on cognitive measures but did have worse outcome on the GOS-E (W=130, p=0.048). Changes in ^{123}I-Ioflupane SBR in the whole striatum and putamen did not relate to cognitive measures.

UPDRS score relates to ^{123}I-Ioflupane scan abnormalities

None of our patients had evidence of clear motor parkinsonism, which is usually the case after single TBI (Krauss and Jankovic, 2002). Despite this, six patients had high UPDRS

motor subscale scores (>9) (Racette *et al.*, 2006) (Fig. 6), which was due to pyramidal or cerebellar signs on examination. Patients with abnormal ^{123}I-Ioflupane clinical reports had significantly higher UPDRS scores than patients with normal reports (W=113, p=0.002). This was also true for those patients with abnormal striatal (W=90.5, p=0.037) or abnormal caudate SBR using the 2 s.d. criteria (W=51, p=0.002). For the caudate, this relationship was also present with the less stringent 1 s.d. criteria (W=151, p=0.027). Putamen SBR did not relate to UPDRS score using either classification (both p>0.2).

Patients showed reduced volume of the substantia nigra and all sub-divisions of the striatum

The substantia nigra was significantly smaller in patients compared to controls (t(51.3)=-3.54, p<0.001) (Fig. 7A). In addition, all three striatal sub-regions were significantly smaller in patients compared to controls; caudate (t(37.69)=-3.45, p=0.001), putamen (t(46.0)=-4.26, p<0.001) and nucleus accumbens (t(49.5)=-4.17, p<0.001).

Substantia nigra volume is related to ^{123}I-Ioflupane specific binding ratios

Substantia nigra volume was related to caudate and putamen SBR. Linear regression showed substantia nigra volume was related to caudate SBR (*b*=3.38, SE=1.63, t=2.07, p=0.04), such that low volume was associated with low SBR (Fig. 7A). There was no significant interaction between group and substantia nigra volume (p=0.76), but the relationship was present when examining the patient group alone (*b*=3.83, SE=1.86, t=2.06, p=0.04). Substantia nigra volume was also related to putamen SBR (*b*=3.28, SE=1.63, t=2.01, p=0.04). Again, there was no interaction between group and substantia nigra volume (p=0.77). Substantia nigra

volume was not related to nucleus accumbens SBR, although the relationship was close to significance (p=0.06). The volumes of the caudate, putamen and nucleus accumbens were not related to their respective SBRs (caudate p=0.58, putamen p=0.26, nucleus accumbens p=0.21).

Patients show damaged nigrostriatal connections to the caudate

We next examined nigrostriatal tract structure, defined from analysis of high-resolution HCP data. Patients showed significantly higher MD in the white matter area through which the nigrostriatal tract passes (t(63.2)=3.39, p=0.001) (Fig. 7B). There was no difference in FA between patients and controls (t(41.6)=-0.69, p=0.494). We explored connections to sub-regions within the striatum. A mixed-effects model showed an interaction between tract and group (F(2,120)=8.7, p<0.001), which resulted from a significantly higher MD in the nigro-caudate tract for patients compared to controls (t(57.9)=3.48, p<0.001) with no difference in the nigro-putamen (p=0.3) or nigro-nucleus accumbens tracts (p=0.2). Analyses of FA in these tracts did not reveal any differences between the groups (nigro-caudate tract, p=0.4; nigro-putamen tract, p=0.08; nigro-nucleus accumbens tract, p=0.3).

Nigrostriatal tract structure is related to substantia nigra volume

Linear regression showed substantia nigra volume was related to MD in the nigrostriatal tract (*b*=-0.31, SE=0.10, t=-3.11, p=0.003), such that low volume was associated with high MD. In contrast, there was no significant relationship between MD in the nigrostriatal tract and striatal SBR (p=0.081) or between MD in the nigro-caudate tract and caudate SBR (p=0.078), although both these relationships approached significance.

The presence of frontal lobe or striatal lesions does not alter striatal SBR

Twenty-three patients had visible frontal lobe lesions on T1 imaging. These patients did not have a significant reduction in SBR in the whole striatum or its sub-regions compared to patients with no frontal lobe lesions and controls. In addition, there was no relationship between the size of frontal lobe lesions and SBR in the whole striatum or its sub-regions. Six patients had visible lesions in their caudate, three in the putamen and two in the nucleus accumbens. Patients with lesions in these structures did not have different SBRs compared to patients without lesions (caudate $p=0.46$, putamen $p=0.97$, nucleus accumbens $p=0.38$).

Discussion

One third of our moderate-severe TBI patients had abnormal striatal DaT on clinical reporting of ^{123}I-Ioflupane SPECT scans. The caudate was affected to a greater extent than other striatal regions. However, at the individual level there was a large degree of variability, with some patients showing clear abnormalities in the other regions of the striatum. DaT abnormalities in the caudate were associated with reduced substantia nigra volume, and there was evidence of nigrostriatal tract damage particularly affecting projections to the caudate. Taken together our results suggest that the dopaminergic system is commonly affected by TBI. The caudate is most commonly affected although all areas of the striatum can be disrupted. Within individuals, the pattern of striatal abnormality is likely to reflect the location of nigrostriatal tract damage produced by axonal and midbrain damage.

Our findings extend the two previous imaging studies in humans demonstrating altered DaT levels in the striatum after TBI (Donnemiller *et al.*, 2000; Wagner *et al.*, 2014). Donnemiller

and colleagues found more than 50% reduction in striatal DaT binding using the SPECT tracer [^{123}I]β-CIT in a group of ten patients who were in a vegetative state or had persisting akinetic-rigid features. Wagner and colleagues studied a less severe group of 12 moderate/severe TBI patients and found a 15% striatal reduction using the PET ligand [^{11}C]β-CFT. Our patients were of similar severity to Wagner's study and we showed a similar magnitude of reduction in DaT (11% for whole striatum). Therefore, the three studies together suggest that reductions in striatal DaT are dependent on injury severity.

Our results are also in line with animal models of TBI, which show reduced DaT expression in the striatum (Yan *et al.*, 2002; Wagner *et al.*, 2005b; Shimada *et al.*, 2014). The DaT resides on presynaptic dopaminergic neurons, therefore loss of dopaminergic fibres results in reduced DaT levels. In addition, synaptic dopamine levels rapidly affect DaT expression (Gulley and Zahniser, 2003). Therefore, the hypodopaminergic state seen post-traumatically will lead to a compensatory down-regulation in DaT expression (Bales *et al.*, 2009). Animal work suggests that functional down-regulation is the main driver of reduced DaT levels post-traumatically as other markers of dopaminergic cell density were unaffected (Wagner *et al.*, 2005b).

Our SPECT results are compatible with functional down-regulation of DaT, but the MRI assessment suggests that dopaminergic cell loss may also be a factor. Reduced substantia nigra volume indicates cell loss in this region, which correlates with striatal DaT binding. This is likely to be at least partly due to direct damage to the cell bodies in the nuclei themselves. Brainstem nuclei are susceptible to the effects of trauma (Adams *et al.*, 1989). Focal injury is often seen in the midbrain and computational models of TBI demonstrate high strain in the midbrain as the brain pivots in this region (Zhang *et al.*, 2001). In addition, neuropathological studies in humans who have suffered repetitive head injuries show gross and microscopic changes to the substantia nigra (Smith *et al.*, 2013).

In addition to direct damage to the midbrain nuclei, dopaminergic neurons may suffer axonal injury to their projecting fibres leading to Wallerian degeneration and subsequent cell death of substantia nigra neurons. Animal models show that substantia nigra damage can be produced as a remote effect of cortical injury (van Bregt *et al.*, 2012). This can result from damage to long-distance axonal projections that are vulnerable to the deforming forces generated by the acceleration and deceleration of the brain during trauma (Gennarelli *et al.*, 1982). The ascending nigrostriatal dopaminergic axons may be particularly vulnerable as they are poorly or unmyelinated (Reeves *et al.*, 2005; Staal and Vickers, 2011). We used high-resolution diffusion MRI data from the HCP to define the locations of the nigrostriatal projections and applied the results to assess nigrostriatal tract damage. A limitation of the diffusion results is that the nigrostriatal tracts cannot be specifically resolved and only make up a small percentage of the fibres in the white matter area studied. However, diffusion abnormalities were seen in the white matter through which the nigrostriatal tract passes. This reduction correlated with substantia nigra volume, suggesting that cell death in this region was linked to axonal injury within the nigrostriatal tract.

A striking feature of our results is that post-traumatic changes in DaT levels were predominantly seen in the caudate. This was not due to focal injury within the caudate, as few patients had visible lesions in their striatum and there was no relationship between lesion presence and DaT SBRs. In addition, we didn't observe a more subtle relationship between the volume of striatal sub-regions and DaT SBRs that would indicate a non-specific effect of atrophy. In contrast, our results provided evidence that caudate DaT might be particularly disrupted because of the high levels of damage within nigrostriatal fibres projecting to the caudate. Taken together the pattern of nigrostriatal tract damage and the relationship between tract damage, substantia nigra volumes and DaT binding suggests that nigrostriatal

projections to the caudate might be particularly vulnerable following TBI and that damage to these projections preferentially reduces caudate dopamine after TBI.

TBI patients with very low levels of caudate DaT showed evidence of processing speed and executive function abnormalities. Similar relationships between dopaminergic function and cognition have been shown in healthy individuals (Mozley *et al.*, 2001), patients with Parkinson's disease (Marie *et al.*, 1999; Muller *et al.*, 2000) and patients with Huntington's disease (Backman *et al.*, 1997). Executive functions and processing speed are thought to rely on intact functioning of cortico-striatal circuitry, which links the frontal cortex and caudate and is mediated by dopaminergic neurotransmission (Divac *et al.*, 1967; Backman *et al.*, 1997). Dopaminergic therapies in Parkinson's disease ameliorate at least some of the associated cognitive impairments (Cools *et al.*, 2002; Mattay *et al.*, 2002). Therefore, TBI patients with evidence of dopaminergic dysfunction may benefit from dopaminergic medications for cognitive impairments and DaT neuroimaging might be a useful way to predict treatment response or stratify treatment selection.

Previous work has shown TBI patients with obvious parkinsonism display striatal DaT abnormalities (Donnemiller *et al.*, 2000). Here none of our patients were parkinsonian, which is the case for the vast majority of TBI patients (Krauss and Jankovic, 2002). Despite the absence of parkinsonism, our patients showed frequent evidence of striatal dopamine abnormalities. The lack of parkinsonism might reflect the location of striatal dopaminergic abnormalities, which were more pronounced in the caudate. Dopaminergic depletion in Parkinson's disease initially occurs in the posterior putamen and reduced DaT levels in this region relate to motor signs (Guttman *et al.*, 1997; Ma *et al.*, 2002). At the group level, our patients did not have a significant reduction of DaT in the putamen. However, some individuals had clear reductions in putaminal DaT levels. This might be due to the severity of dopaminergic changes. At the time of symptom onset in Parkinson's disease there is usually

over 50% reduction in DaT levels (Cheng *et al.*, 2010). None of our patients had reductions in DaT to this degree, therefore the impact of TBI on the dopaminergic system maybe below the threshold required to display overt signs of parkinsonism. However, TBI patients with evidence of reductions in DaT levels might be predisposed to the future development of parkinsonism. Our work does not provide information about the long-term significance of reduced DaT, which is a limitation of the cross-sectional study design. Further longitudinal studies will be needed to inform how these abnormalities evolve and to what extent DaT abnormalities are a risk factor for late deterioration.

Our in vivo imaging assessment uses a semi-quantitative technique, which assumes that non-specific uptake of ^{123}I-Ioflupane is equivalent throughout the brain. Therefore, if TBI caused variable changes to this non-specific uptake in different brain regions (e.g. through changes to striatal absorption of ^{123}I-Ioflupane) this could bias the results. We have no reason to suspect distinct non-specific uptake across the striatum to explain the pattern of abnormalities we observed and our results are also in line with animal models of TBI using different techniques, which also show reduced DaT expression in the striatum (Yan *et al.*, 2002; Wagner *et al.*, 2005b; Shimada *et al.*, 2014).

In conclusion, striatal DaT abnormalities measured using ^{123}I-ioflupane SPECT were commonly seen following moderate/severe TBI. One third of the SPECT scans were clinically reported as abnormal. These changes are a marker of reduced striatal dopamine. Our results suggest this is due to the pattern of nigrostriatal tract damage produced by axonal injury. Cognitive impairment was greater in patients with marked reductions in caudate DaT and similar cognitive impairments respond to dopaminergic treatments in other contexts. Therefore, DaT abnormalities following TBI may assist treatment selection after TBI.

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Figure Legends:

Figure 1. Methods: A. (i) 123I-Ioflupane SPECT scan analysis: Individual reconstructed ^{123}I-Ioflupane SPECT scans were co-registered with their native T1 MRI using rigid transformation. T1 images were warped to an average group template using a diffeomorphic nonlinear registration (DARTEL). Templates were registered to Montreal Neurological Institute 152 (MNI) space. Then the individual flow-fields and template transformation from DARTEL were applied to the SPECT images to produce MNI space images, without modulation. (ii) Schematic of a dopaminergic synapse illustrating the role of the pre-synaptic DaT in re-uptaking synaptic dopamine: DOPA=L-3,4-dihydroxyphenylalanine, DA=Dopamine, nvDA=Non vesicular dopamine, VMAT2=Vesicular monoamine transporter 2, DAT=Dopamine transporter, TH=Tyrosine hydroxylase, L-AAD=L-amino acid decarboxylase,**B.** Volumetric MRI analysis: T1 images were warped to MNI space (as above) but including modulation by the Jacobian determinants derived from DARTEL. A mask of the substantia nigra and striatum in standard space were used to calculate volumes for these structures in each individual. **C.** Diffusion MRI analysis: (i) Making the nigrostriatal mask: Fibre-tracking using the MRtrix package was performed on 100 subjects from the Human Connectome Project (HCP) to create binarised masks for the nigro-striatal tract as a whole, as well as the sub-regions; nigro-caudate, nigro-putamen and nigro-nucleus accumbens tracts;

(ii) Processing diffusion data: Diffusion-weighted images were preprocessed using standard methods and tensor-based registration performed using DTI-TK.

Figure 2. Lesion map: Thirty patients had focal lesions visible on T1 MRI. These were overlaid to produce a lesion map. Maximal areas of overlap of lesions were seen in the anterior frontal and temporal lobes. There were no lesions in the substantia nigra (light blue) and minimal lesion load in the striatum (green) and in the area through which the nigrostriatal tract passes (dark blue).

Figure 3. 123I-ioflupane SPECT scan examples: A selection of 16 TBI patients and 3 controls. Subject's age, gender (M/F) and the time since injury in months is shown. The colour scale shows the 123I-Ioflupane SBR.

Figure 4. Clinical ^{123}I-ioflupane SPECT scan reports and quantitative comparison: Comparison between individual clinical reporting and quantitative assessment of ^{123}I-ioflupane SPECT scans: Each line represents a single subject. Red signifies 'abnormal' and blue 'normal'. For the quantitative assessment in the whole striatum, caudate and putamen two criteria were used; greater than 1 and greater than 2 standard deviations standard deviation (SD) below the mean SBR in the control group.

Figure 5. 123I-ioflupane SPECT scan results: A. Results of the voxelwise analysis: areas of significant reduction of ^{123}I-Ioflupane SBR are shown in red/yellow. The striatal mask is shown for comparison in blue. The colour bar shows the corrected p-values. **B.** Anatomical location of the caudate, putamen and nucleus accumbens for reference. **C.** Region of interest analyses: Plots show ^{123}I-Ioflupane SBR corrected for age for patients and controls in the caudate, putamen and nucleus accumbens. The SBR corrected for age are the residuals from the linear regression of age on SBR. ** Signifies p=0.001.

Figure 6. Characteristics of patients with UPDRS motor sub-score greater than nine: Clinical details of the six patients with UPDRS scores higher than nine. Examination findings are included along with a functional outcome measure (GOS-E), UPDRS score and an axial slice of their 123I-Ioflupane scan.

Figure 7. Volumetric and diffusion tensor MRI results: A. Volumetric assessment: Top line of plots shows volume corrected for intracranial volume for patients and controls in the substantia nigra, caudate, putamen and nucleus accumbens. Bottom two plots show ^{123}I-Ioflupane SBR of the caudate and putamen (corrected for age) correlate positively with substantia nigra volume (corrected for age and intracranial volume). Patients are in red and controls in blue. The SBR and volumes corrected for age are the residuals from the linear regression of age on SBR and volume. Key at the bottom shows masks used for volumetric analysis of the different regions. **B.** Diffusivity assessment: Top line of plots shows mean diffusivity corrected for age for patients and controls in the nigro-striatal, nigro-caudate, nigro-putamen and nigro-nucleus accumbens tracts. Bottom two plots show ^{123}I-Ioflupane SBR of the whole striatum and caudate (corrected for age) correlate negatively with the mean diffusivity of the nigro-striatal and nigro-caudate tracts (corrected for age) respectively. Patients are in red and controls in blue. ** Signifies $p<0.01$, SBR=specific binding ratio, r_s=Spearman's rho, MD=Mean diffusivity**.** The SBR and mean diffusivities corrected for age are the residuals from the linear regression of age on SBR and mean diffusivity. Key at the bottom shows the masks used for the nigro-putamen, nigro-caudate and nigro-nucleus accumbens tracts (the nigro-striatal tract was all three combined).

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